Assessment of Tissue Viability Following Electroosmotic Push-Pull Perfusion from Organotypic Hippocampal Slice Cultures

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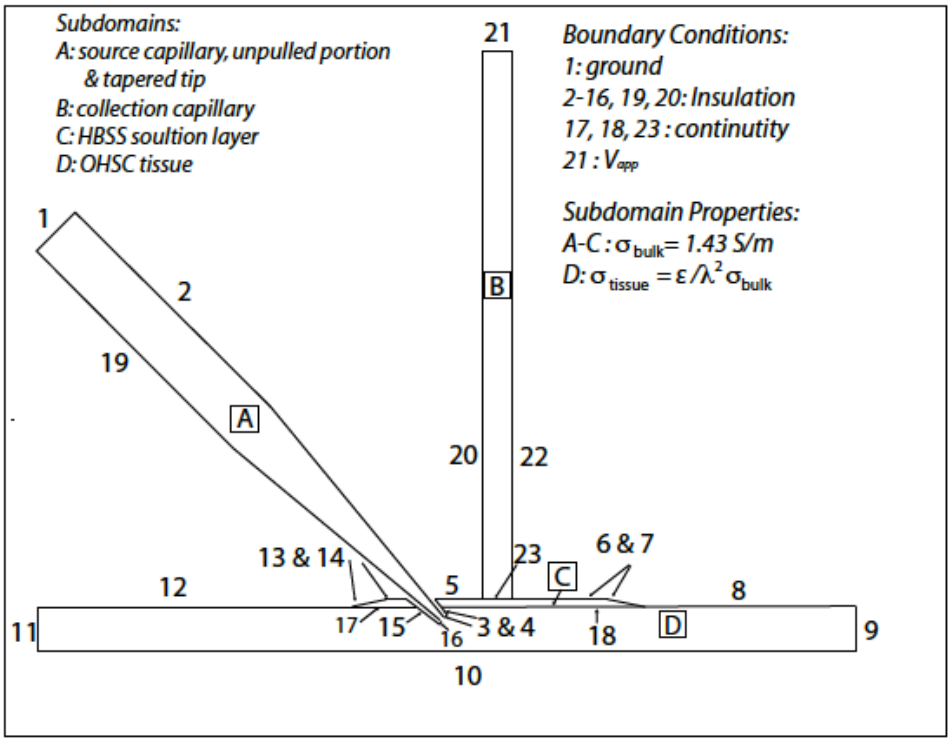
Supporting Information


Subdomains:
A: source capillary, unpulled portion & tapered tip
B: collection capillary
C: HBSS soultion layer
D: OHSC tissue
Boundary Conditions:
1: ground
2-16, 19, 20: Insulation
17, 18, 23 : continutity
21 : V_{app}
Subdomain Properties:
A-C : σ_{bulk}= 1.43 S/m
D: $\sigma_{tissue} = \varepsilon/\lambda^2 \sigma_{bulk}$
1
2
19
A
21
B
20
22
23
C
6 & 7
8
9
10
11
12
13 & 14
5
17
15
16
3 & 4
18
D

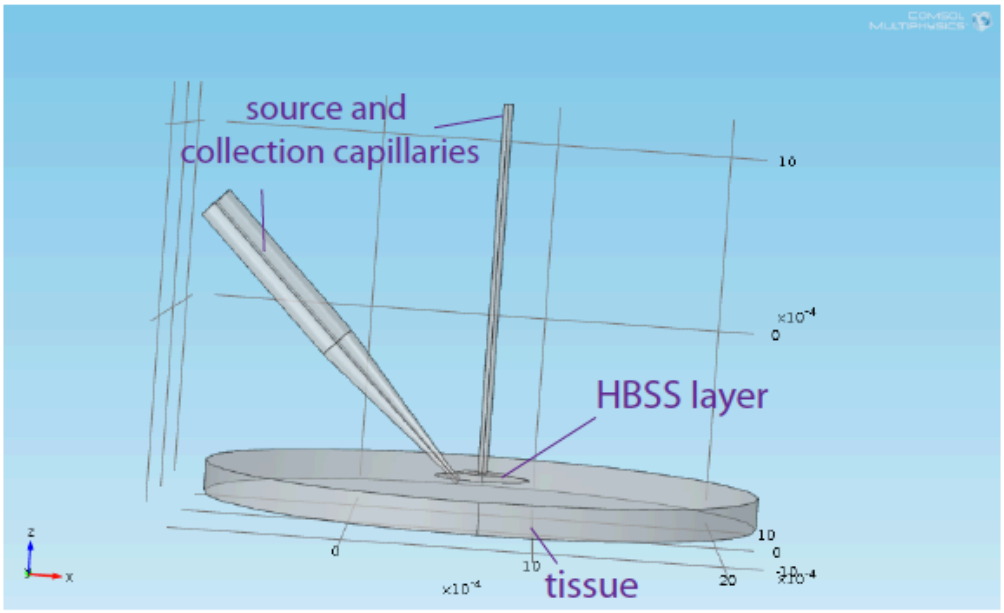

source and collection capillaries
HBSS layer
tissue

Figure SI- 1. COMSOL model of push-pull electroosmotic sampling.

(TOP) This model was constructed with shortened source and collection capillaries. The collection capillary (B) is 1 mm. The source (A) is a combination of two 1 mm segments representing the main part of the capillary and the tapered portion. The tapered tip of the source capillary is 1 mm long. It is inserted into the tissue at a 45° angle, measured from the tissue surface. The thickness of the HBSS buffer (C) layer is as thick as the experimental capillary-to-tissue distance (CTD). The outer diameters of both source and collection capillaries are not shown but have no bearing on the actual model except to create an empty space between the inner diameter of the source capillary and the tissue. The tissue (D) is represented by a disk of thickness is 168.5 μm with the capillaries placed in the center. (BOTTOM) Model of push pull electroosmotic sampling in COMSOL 3D workspace. This figure is also found in the supporting information of the companion paper.[1]

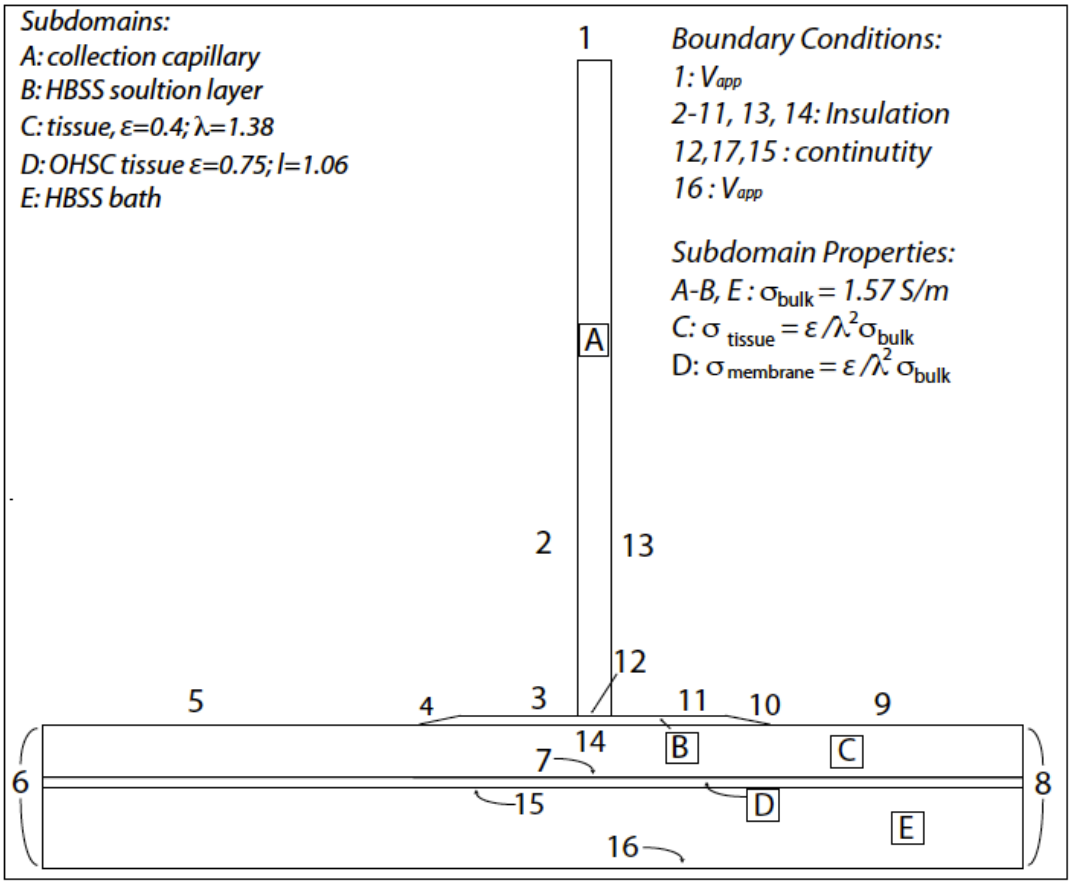


Figure SI- 2. Schematic for the COMSOL model of single capillary EO sampling.

The single capillary electroosmotic sampling model was constructed with a shortened collection capillary (A, 1 mm). The thickness of the HBSS buffer (B) layer is as thick as the experimental CTD and has a conductivity of 1.57 S/m (for in-house prepared HBSS solution). The outer diameter of the collection capillary is not shown, and has no bearing on the electric field distribution in the model. The tissue (C) is represented by a disk of thickness is 168.5 μm with the collection capillary placed in the center. The tissue has a porosity (ε) of 0.4 and a tortuosity (λ) of 1.38. The insert membrane below the tissue (D) is 28 μm thick, with a porosity of 0.75 and a tortuosity of 1.05. Below the insert membrane is a subdomain (E) that represents the HBSS bath. It is not drawn to scale here but is 5 mm thick. V_{app} is the total applied field divided by 1 mm (i.e. 1000 V applied over 30 cm = 300 V/cm x 1 mm = 3.33 V for this simulated model).

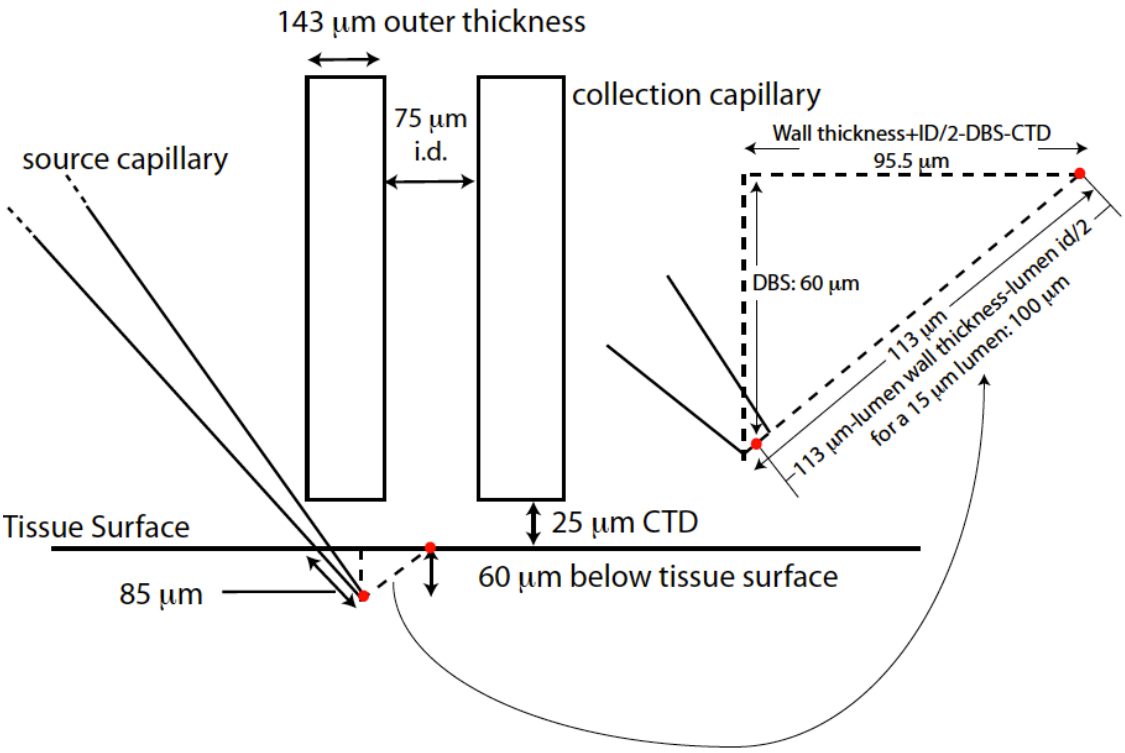


Figure SI- 3. The geometry of capillary placement

Push-pull sampling geometry when the source capillary is inserted 60 μm below the tissue surface (DBS) and the collection capillary is raised 25 μm above the tissue surface (CTD). The central axis of the source capillary is at a 45° angle from the tissue surface. The distance between the source tip and the tissue surface under the center of the collection lumen (indicated by red dots) can be calculated as shown to the right side of the figure. This figure is also found in the supporting information of the companion paper.[1]

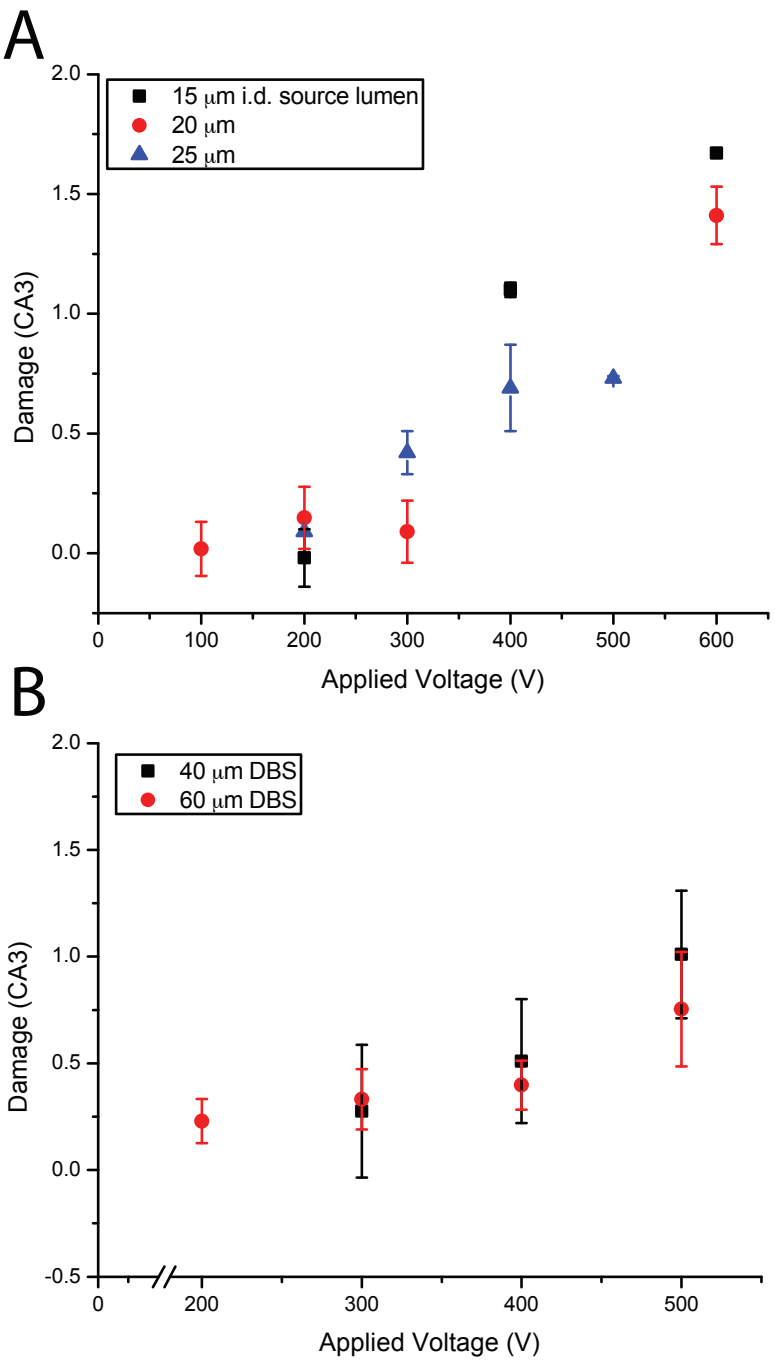


Figure SI-4. Effect of source tip size and insertion depth on damage

These graphs illustrate that there is no dependence of (A) source capillary tip size or (B) insertion depth (DBS) on damage trends.

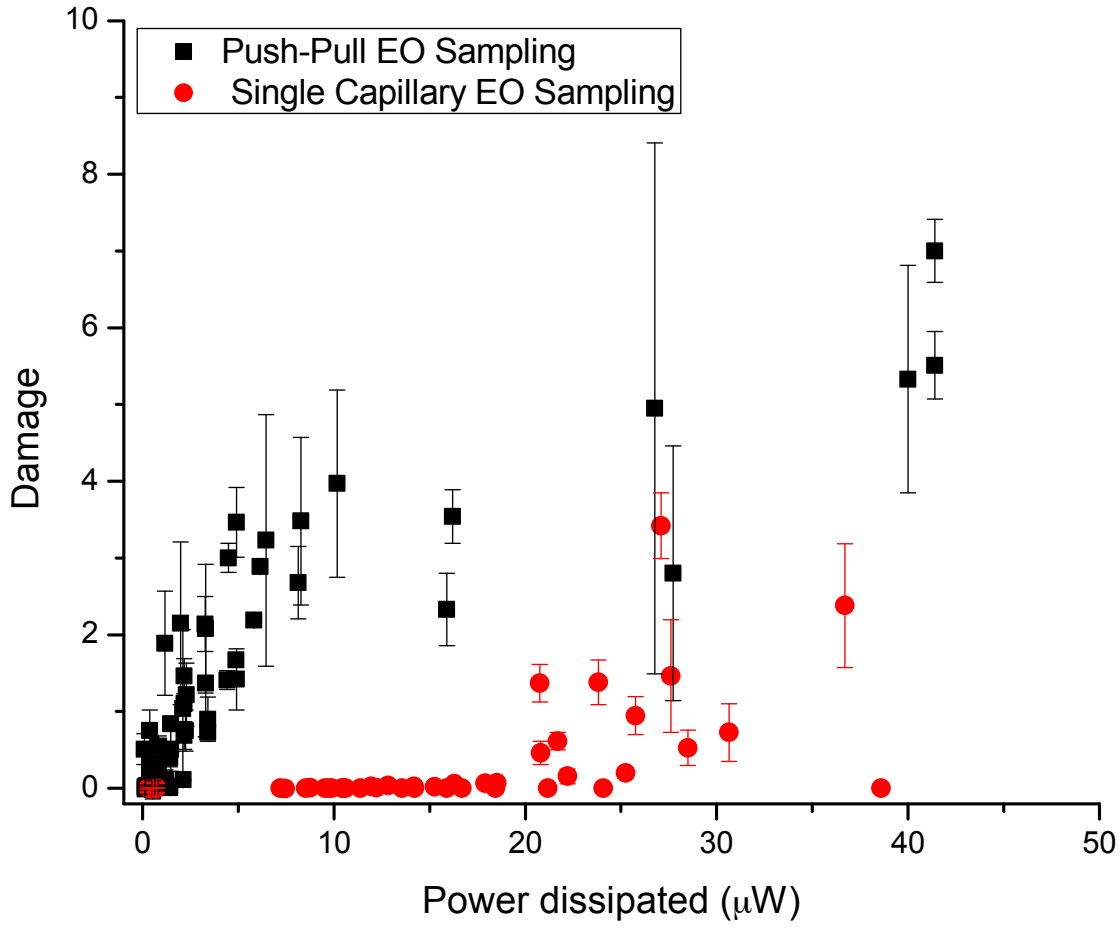


Figure SI-5. The difference in damage trends between the EOPPP and the single capillary sampling model.

Power is calculated by integrating the electric field over the entire tissue subdomain ((D) in Fig. SI-4 and (C) in Fig. SI-5) and multiplying by the conductivity of the tissue.

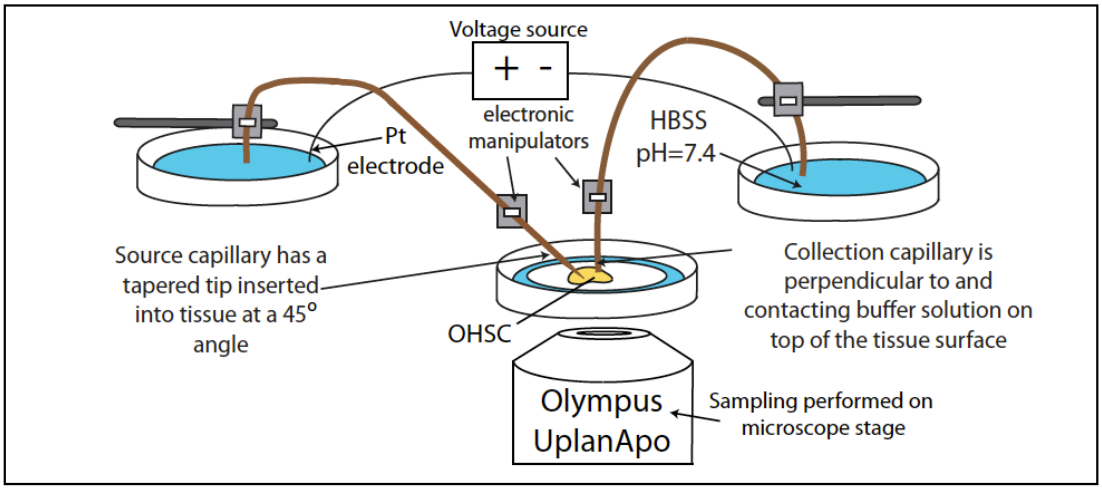


Figure SI-6. Push-pull electroosmotic sampling.

The OHSC tissue sits on a PTFE porous support over HBSS solution. The source capillary is a 30 cm fused silica capillary with a tapered (15-30 μm) tip, with tip inserted at a 45° angle into the tissue. A second 30 cm fused silica capillary is positioned perpendicular to the tissue surface with lumen in contact with the tissue through a thin (15-50 μm) layer of HBSS. The distal (non-tissue) ends of each capillary are submerged in dishes containing HBSS. These dishes are connected to a power source by platinum electrodes.

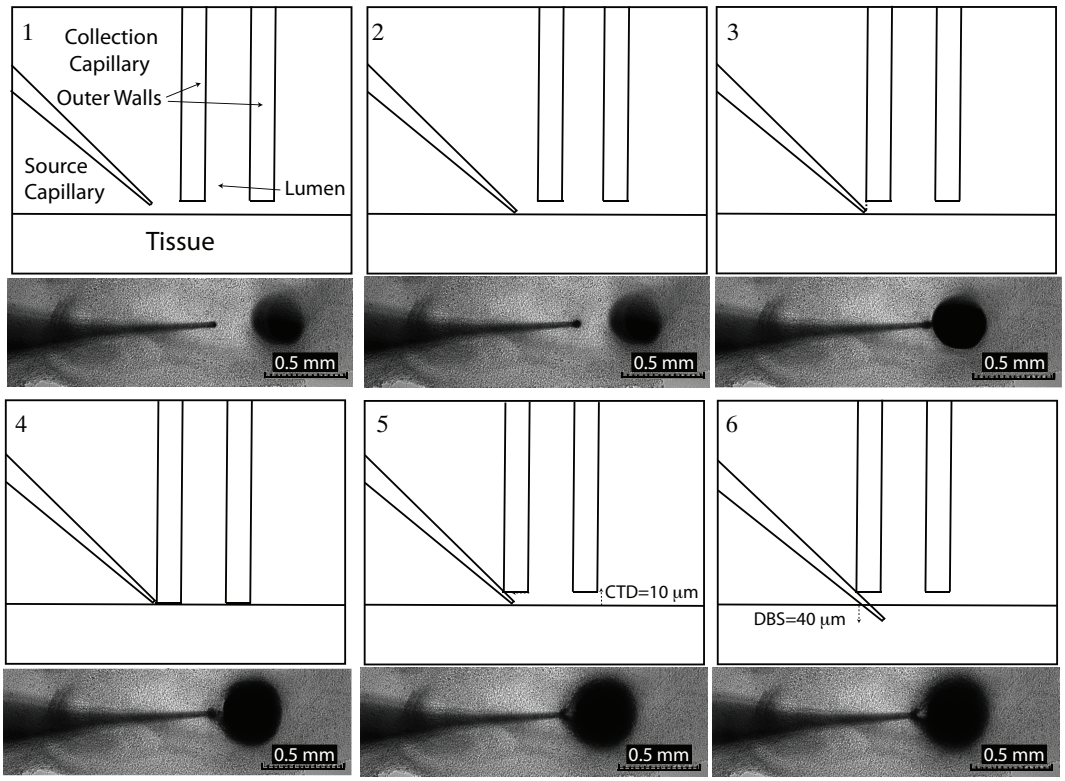


Figure SI-7. Capillary positioning.

The top portion of each panel is a sketch of the capillaries from the side. The bottom portion is the actual view from below (microscope image). Positioning is accomplished as follows: (1) Capillaries have been trimmed, filled, and are above the tissue surface ready to be positioned. (2) The source capillary was lowered at a 45° angle to the surface of the tissue until it just touched the tissue. (3) The collection capillary was lowered to the point where its shadow has crisp edges. The edge of the middle of shadow was lined up and touches the source capillary lumen shadow where it had touched the tissue surface. (4) The collection capillary was lowered until it just barely touched the surface. At this point, the edge of the tapered tip and the center of the outside edge of the collection capillary lumen were just barely touching each other and the surface of the tissue. (5) The collection capillary was raised a desired distance (capillary – tissue distance, CTD) and then moved towards the source capillary that same distance (this move was first designed to keep the capillaries as close as possible, and retained for all sampling to keep a standard procedure.) (6) The source capillary was inserted into the tissue a desired distance (distance beneath surface, DBS). This figure is also found in the supporting information of the companion paper.[1]

References

1. Rupert, A. E., Ou, Y., Sandberg, M., and Weber, S. G. (submitted) Electroosmotic push-pull perfusion: description and application to qualitative analysis of the hydrolysis of exogenous galanin in organotypic hippocampal slice cultures, *ACS Chem. Neurosci*.